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Identification and Distribution of Insertion Sequences of *Paracoccus solventivorans*

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Three novel insertion sequences (ISs) (IS*Pso1*, IS*Pso2*, and IS*Pso3*) of the soil bacterium *Paracoccus solventivorans* DSM 11592 were identified by transposition into entrapment vector pMEC1. IS*Pso1* (1,400 bp) carries one large open reading frame (ORF) encoding a putative basic protein (with a DDE motif conserved among transposases [Tnps] of elements belonging to the IS*256* family) with the highest levels of similarity with the hypothetical Tnps of *Rhodospirillum rubrum* and *Sphingopyxis macrogoltabida*. IS*Pso2* (832 bp) appeared to be closely related to IS*Ppa2* of *Paracoccus pantotrophus* DSM 11072 and IS*1248* of *Paracoccus denitrificans* PdX22, both of which belong to the IS*427* group (IS*5* family). These elements contain two overlapping ORFs and a putative frameshift motif (AAAAG) responsible for production of a putative transframe Tnp. IS*Pso3* (1,286 bp) contains a single ORF, whose putative product showed homology with Tnps of ISs classified as members of a distinct subgroup of the IS*5* group of the IS*5* family. The highest levels of similarity were observed with IS*Ssp126* of *Sphingomonas* sp. and IS*1169* of *Bacteroides fragilis*. Analysis of the distribution of ISs of *P. solventivorans* revealed that IS*Pso2*-like elements are the most widely spread of the elements in nine species of the genus *Paracoccus*. IS*Pso1* and IS*Pso3* are present in only a few paracoccal strains, which suggests that they were acquired by lateral transfer. Phylogenetic analysis of Tnps of the novel ISs and their closest relatives showed their evolutionary relationships and possible directions of lateral transfer between various bacterial hosts.

The classification of the genus *Paracoccus* (alpha subgroup of the *Proteobacteria*) has undergone serious changes during the past decade. Several new species have been isolated, and the status of other species has been reevaluated. Currently, the genus consists of 17 species, which are found in different environments. Some of these species, including *Paracoccus alcaliphilus* (38) *P. carotinifaciens* (36), *P. aminophilus*, *P. aminovorans* (37), and *P. kondratievae* (11), were isolated from soil. Other species were isolated from environments containing a range of toxic components; e.g., *P. alkenifer* was isolated from biofilters used in the treatment of waste gases from an animal rendering plant (22), *P. methylutens* was isolated from groundwater contaminated with dichloromethane (12), *P. pantotrophus* was isolated from a sulfide-oxidizing, denitrifying fluidized-bed reactor in a plant (30), and *P. kocurii* was isolated from wastewater from semiconductor manufacturing processes (26). Some strains of *P. denitrificans*, which was the first *Paracoccus* species isolated (4), have also been found in a number of different habitats, including sewage, sludge, horse manure, and cow dung (20), as well as in soil. Bacteria belonging to the genus *Paracoccus* are probably also important components of many wastewater treatment system communities (25). The number of known habitats for bacteria belonging to the genus *Paracoccus* has expanded. Two new species were isolated recently from the marine environment; *P. seriniphilus* was isolated from the marine bryozoan *Bugula plumosa* (27), and *P. zeaxanthinifaciens* was isolated from seaweed from the coast of the African Red Sea (5). Furthermore, the first paracoccal species associated with human infection (*P. yeei*) was isolated from the dialysate of a patient with peritonitis (8). These bacteria thus appear to be more widespread than was previously thought.

Paracocci are among the most metabolically versatile bacteria. They are chemoorganoheterotrophs (utilizing a wide variety of organic compounds, including potentially polluting compounds, like acetone) or facultative chemolitoautotrophs (utilizing reduced sulfur compounds, such as sulfides, thiosulfate, thiocyanate, or molecular hydrogen, as energy sources). Methylotrophy is often observed (with such methyl compounds as formate, methanol, trimethylamine, tetramethylammonium), as is growth in anaerobic conditions (nitrate respiration) (1). Because of their versatile metabolism paracocci can play an important role in the cycling of elements in the environment.

It seems that all these physiological properties raise the possibility that *Paracoccus* species could be used in bioremediation systems, particularly since most species can use nitrate as an alternative electron acceptor. One of these species is *P. solventivorans*, which was first isolated at the site of a natural gas company as an acetone-degrading, nitrate-reducing bacterium (33). The second isolate of this species, DSM 11592 (used in this study), was found in biofilters used for the treatment of waste gases (22). So far, nothing is known about the genetics of *P. solventivorans.* It is only known that strain DSM 11592 carries a 5-kb plasmid, pSOV1 (2).

We initiated studies on transposable elements of *Paracoccus* spp. Such elements significantly enhance the variability and consequently the adaptative capacities of their hosts (7). By using the specific paracoccal entrapment vector pMEC1, we

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TABLE 1. Distribution of IS*Pso2*-related elements in paracoccal strains

Strain	Reference	Hybridization signal with IS*Pso2* as a probe	PCR product with primers specific for:		
			IS*Pso2*	IS*Ppa2*	IS*1248*
P. solventivorans DSM 11592	22	+	+	−	−
P. alcaliphilus JCM 7364	38	+	−	−	−
P. alkenifer DSM 11593	22	+	−	−	−
P. aminophilus JCM 7686	37	+	−	−	−
P. aminovorans JCM 7685	37	+	−	−	−
P. denitrificans DSM 413	28	+	−	−	+
P. denitrificans LMD 22.21	28	+	−	−	+
P. pantotrophus DSM 65	28	+	−	+	+
P. pantotrophus LMD 82.5	28	+	+	−	+
P. pantotrophus DSM 11072	18	+	−	+	+
P. pantotrophus DSM 11073	18	+	−	+	+
P. methylutens DM12	11	+	−	+	+
P. thiocyanatus IAM 12816	19	+	−	−	−
P. versutus UW1	34	+	−	−	−

previously identified and characterized several insertion sequences (ISs) and transposons of different strains of *P. pantotrophus* (3). This analysis allowed us to distinguish transposable elements common in this species (e.g., IS*Ppa2* of the IS*5* family and IS*Ppa5* of the IS*66* family), as well as strain-specific elements (e.g., IS*Ppa1* of the IS*256* family and Tn*3434* of the Tn*3* family). In order to study the occurrence and frequency of lateral transfer in this group of bacteria, we performed studies to identify and determine the distribution of transposable elements of other paracoccal strains. Here we describe molecular characteristics of ISs caught by pMEC1 in *P. solventivorans* DSM 11592. These ISs may be used for construction of novel tools for transposon mutagenesis, which may be very useful for genetic analysis of this interesting group of bacteria.

MATERIALS AND METHODS

Bacterial strains, plasmids, and culture conditions. Strains of *Paracoccus* spp. (Table 1) were grown in Luria-Bertani (LB) medium (31) at 30°C, and *Escherichia coli* strains were grown at 37°C. When necessary, the medium was supplemented with antibiotics as follows: kanamycin, 50 μg/ml; rifampin, 50 μg/ml; and tetracycline, 2 μg/ml.

DNA manipulations. Plasmid DNA was isolated as described by Birnboim and Doly (6). Cloning experiments, digestion with restriction enzymes, ligation, and agarose gel electrophoresis were performed by using standard procedures, as described previously (31). For Southern hybridization (31) DNA probes were labeled with digoxigenin (Roche). Equal amounts of total genomic DNAs from paracoccal strains were digested to completion with appropriate restriction endonucleases and separated by electrophoresis by using 0.8% agarose gels. DNA was blotted onto BioBond-Plus nylon membranes (Sigma) and hybridized under high-stringency conditions (5× SSC [1× SSC is 150 mM NaCl plus 15 mM sodium citrate, pH 7], 1% blocking reagent [Roche], 0.1% *N*-lauroylsarcosine, 0.2% sodium dodecyl sulfate [SDS]) at 68°C overnight. The filters were washed twice in 2× SSC–0.1% SDS at room temperature and twice in 0.1× SSC–0.1% SDS at 65°C.

Transformation. Competent cells of *E. coli* TG1 were prepared and transformed as described by Kushner (21).

Triparental mating. Overnight cultures of donor strain *E. coli* TG1 carrying a mobilizable vector, a *P. solventivorans* recipient strain, and *E. coli* DH5α carrying the helper plasmid pRK2013 (10) were spun down at the maximum speed for 2 min at 4°C in a microcentrifuge and washed twice with LB medium to remove the antibiotics. The donor, recipient, and helper were mixed (1:2:1), and a 100-μl sample of the mixture was spread on a plate containing solidified LB medium. After overnight incubation at 30°C, the bacteria were washed off the plate, and suitable dilutions were plated on selective media containing rifampin (selective marker of the recipient strain) and kanamycin to select transconjugants. Spontaneous resistance of the recipient strains to kanamycin was undetectable under these experimental conditions.

Isolation of insertion mutants. The entrapment vector pMEC1 was introduced into a recipient *P. solventivorans* strain by triparental mating. An overnight culture of a Km^r transconjugant, carrying pMEC1, was spread on plates containing solidified LB medium supplemented with tetracycline. Appropriate dilutions of the culture were also spread on LB medium lacking tetracycline in order to determine the frequency of transposition. Spontaneous resistance of the strain to tetracycline was undetectable under these experimental conditions.

PCR amplification. For amplification of transposable elements captured in pMEC1, five nested pairs of cartridge-specific forward and reverse primers were used as described previously (3). For differentiation of IS*Pso2* and the related element IS*Ppa2*, as well as IS*1248*, in various strains of *Paracoccus* the following pairs of forward and reverse primers were used: LPSO2 (5′-AGGATGCATTG ATTTCTGTT-3′) and RPSO2 (5′-ATAACCAATAGATGACGAGA-3′); LPPA2 (5′-AGGATGCATTGATTTTCGAC-3′) and RPPA2 (5′-ATAACCAG TAGATGACGACC-3′); and L1248 (5′-CAGGATGCATTGATTTTCAG-3′) and R1248 (5′-ATAACCAATAAATGACGGTT-3′). Amplification was performed with a Mastercycler (Eppendorf) by using the synthetic oligonucleotides described above, *Taq* polymerase from Qiagen (supplied with buffer), and appropriate template DNAs. Each 50-μl reaction mixture for amplification contained 2 mM $MgCl_2$, each deoxynucleoside triphosphate at a concentration of 200 μM, 50 pmol of each primer, 0.5 U of *Taq* polymerase, and 1× Q solution (Qiagen). The amplification program was 96°C for 5 min, followed by 35 cycles of 48°C for 30 s, 72°C for 1 min, and 94°C for 1 min; the last cycle was followed by an additional annealing step and a final 10-min extension step.

DNA sequencing and analysis. The nucleotide sequence was determined by using a terminator sequencing kit and an automatic sequencer (ABI 377; Perkin-Elmer). The transposable elements (present in pMEC1 derivatives) were sequenced first with the appropriate sets of cartridge-specific starters (3) and then with primers complementary to the previously determined sequence. Sequence analysis was done with programs included in the Wisconsin Genetic Computer Group Sequence Analysis Software Package, version 8.1 (9). Comparison searches were performed with IS Finder (http://www-is.biotoul.fr/is.html) and with the BLAST program provided by the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/BLAST). Phylogenetic analysis was performed by using the parsimony method (DNAPARS in the software package PHYLIP, version 3.57c) (14), as well as the programs SEQBOOT, CONSENSE, and DRAWTREE to perform bootstrap analysis.

Nucleotide sequence accession numbers. Nucleotide sequences determined in this study have been deposited in the GenBank database under accession numbers AY237733 (IS*Pso1*), AY237732 (IS*Pso2*), and AY311510 (IS*Pso3*).

RESULTS AND DISCUSSION

Identification of ISs in *P. solventivorans*. To identify the ISs of *P. solventivorans*, we used the previously described (3) mobilizable entrapment *E. coli-Paracoccus* shuttle vector pMEC1 (Km^r). This vector carries a selective cartridge (constructed by Schneider et al. [32]), composed of a silent *tetA* gene under control of the *pR* promoter of bacteriophage λ and the gene coding for the λ CI repressor. Inactivation of the repressor gene (e.g., through insertion of an IS) results in constitutive expression of tetracycline resistance. The pMEC1 vector was introduced into *P. solventivorans* DSM 11592, and Tc^r clones were selected as described in Materials and Methods. Tc^r mutants appeared with a frequency of 2.9×10^{-5}. We tested 100 Tc^r colonies for plasmid content. Most of the plasmids tested (92%) carried inserts that were smaller than 2 kb, while 8% were the size of pMEC1. Restriction analysis followed by hybridization analysis (with digoxigenin-labeled internal fragments of randomly chosen inserts as probes) revealed the presence of three different classes of elements caught in pMEC1 (0.8, 1.2, and 1.4 kb) (data not shown). To localize the insertion sites of the elements, we used five sets of previously described cartridge-specific primers (3) together with pMEC1-derived plasmids (as template DNAs) in PCRs. We amplified all the

TABLE 2. Characteristic features of ISs of *P. solventivorans*

IS	Length (bp)	G+C content (mol%)	IR length (bp)/no. of mismatches[a]	DR length (bp) (sequence)	No. of ORFs	IS family/IS group
IS*Pso1*	1,400	67	28/5	8 (ATCACCTT)	1	IS*256*
IS*Pso2*	832	60	14/1	2 (TA)	2	IS*5*/IS*427*
IS*Pso3*	1,283	62	11	4 (TAAA)	1	IS*5*/IS*5*

[a] Number of mismatches between two IRs of a given IS.

inserts, which confirmed that the insertions were within the *cI* gene (data not shown). The representative elements of each of the classes distinguished were sequenced. A comparison with the nucleotide sequences in databases revealed that these sequences were novel ISs, and they were designated IS*Pso1*, IS*Pso2*, and IS*Pso3*. The G+C contents of the sequences identified were in the range from 60 to 67 mol% (Table 2), while that of *P. solventivorans* total DNA was 68.5 to 70 mol% (1).

Structural analysis of IS*Pso1*, *ISPso2*, and IS*Pso3*. The genetic organization of the three ISs of *P. solventivorans* DSM 11592 is typical of the genetic organization of the majority of known ISs, since they contain an open reading frame(s) (ORF) for transposase (Tnp) and terminal inverted repeated sequences (IRs). Moreover, they are flanked by direct repeats (DRs) of the target sequence, which are generated upon insertion. The Tnps encoded by all these elements contain three domains (designated N2, N3, and C1), which have three conserved residues (two aspartate [D] residues and one glutamate [E] residue). These residues constitute the DDE motif (so-called catalytic triad) typical of many bacterial Tnps (7). The spacing between these residues, as well as the presence of other conserved residues within the domains, is different in different IS families or groups (7).

IS*Pso1* (1,400 bp) carries one large ORF (1,197 bp) (ORF1) encoding a putative basic protein (pI 9.25) consisting of 398 amino acids and having a predicted molecular mass of 44.65 kDa. The ORF1 product contains the DDE motif, in which the acidic residues are separated by 65 and 105 amino acids, respectively (Fig. 1); this motif is highly conserved among elements belonging to the IS*256* family (23). Additionally, the nucleotide sequences of the IRs of IS*Pso1* (Fig. 2A), as well as the size (8 bp) of the DRs resulting from its transposition (Table 2), are also typical of the members of this family (7). Database comparison searches revealed that the predicted translation product of ORF1 exhibited the highest levels of similarity with a hypothetical protein (product of the *rrub3476* gene) of *Rhodospirillum rubrum* (accession no. ZP 00016443) (65% identity and 72% similarity) and with TnpA of *Sphingopyxis macrogoltabida* (accession no. BAB07803) (57% identity and 67% similarity), both of which exhibit similarity to Tnps encoded by members of the IS*256* family. All these data show that IS*Pso1* can be classified as a new member of the IS*256* family.

Another element, IS*Pso2* (832 bp), appeared to be highly homologous (87% identity at the nucleotide sequence level) to the previously described IS*Ppa2* of *P. pantotrophus* DSM 11072 (3) and IS*1248* of *P. denitrificans* PdX22 (39), both of which are members of the IS*427* group (IS*5* family). Moreover, all these

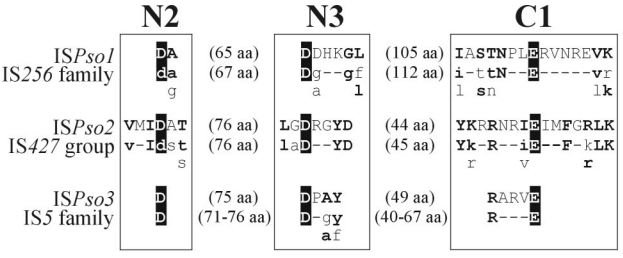

FIG. 1. Comparison of the amino acid sequences of the predicted DDE motifs of the putative Tnps encoded by IS*Pso1*, IS*Pso2*, and IS*Pso3* with the appropriate family- and group-specific consensus sequences (23). In the consensus sequences uppercase letters indicate conservation within the family, lowercase letters indicate predominant amino acids, and dashes indicate nonconserved residues. Residues forming the DDE motif are indicated by a black background. The N2, N3, and C1 domains are enclosed in boxes and labeled. The residues conserved in the domains of the Tnps analyzed and the consensus sequences are indicated by boldface type. The numbers in parentheses are the distances (in amino acids [aa]) between the residues forming the DDE motif.

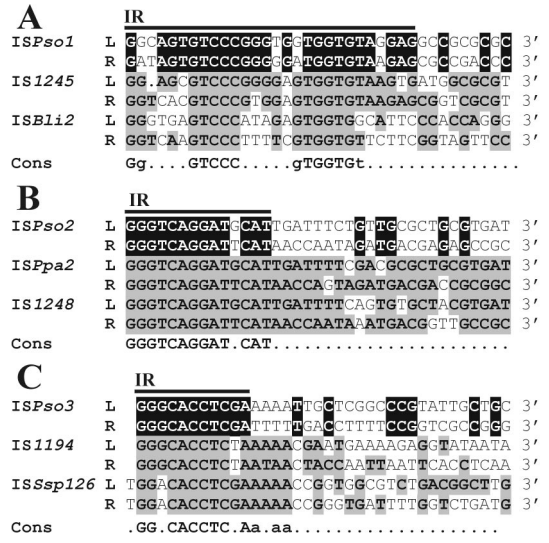

FIG. 2. Alignment of the terminal nucleotide sequences of IS*Pso1* (A), IS*Pso2* (B), and IS*Pso3* (C) and their relatives. The identical residues of the termini of an IS of *P. solventivorans* are indicated by a black background. The putative IRs are indicated by bars. The nucleotides of other ISs identical to those of IS*Pso1* (IS*1194* [accession no. Y13626] and IS*Bli2* [accession no. AF195203]), IS*Pso2* (IS*Ppa2* [accession no. AY179508] and IS*1248* [accession no. PDU08856]), or IS*Pso3* (IS*1246* [accession no. NC 003350] and IS*Ssp126* [accession no. SSP277295]) are indicated by boldface type and a gray background. The consensus sequence (Cons) of the IRs compared is included in each panel. L, sequences at the 5′ (left) end; R, complementary sequences at the 3′ (right) end of the elements.

elements have the same structure, since they contain two overlapping ORFs (ORF1 [347 bp] and ORF2 [569 bp]) and nearly identical IRs (Fig. 2B). Within the ORF1-ORF2 overlap (159 bp) there is a putative frameshift motif (AAAAG), which was shown to promote generation of fusion Tnps in some IS*1* and IS*3* family members (13, 16, 41). The putative fusion protein encoded by IS*Pso2*, whose predicted molecular mass is 28.8 kDa and whose pI is 10.74, has a DDE motif (Fig. 1) that matches the consensus sequence determined for the IS*427* group products (23). The levels of identity and similarity between the corresponding ORFs of IS*Pso1* and ORFs of IS*Ppa2* or IS*1248* were approximately 90 and 94%, respectively, for ORF1 and 85 and 88%, respectively, for ORF2. However, on the basis of the classification guidelines, the levels of identity below 95% for amino acid sequences and below 90% for DNA sequences allowed classification of these ISs as different, closely related elements (24).

Analysis of the nucleotide sequence of IS*Pso3* (1,286 bp) revealed the presence of one major ORF (ORF1), spanning 84% of the element. ORF1 encodes a putative peptide consisting of 374 amino acids with predicted molecular mass of 39.4 kDa and a pI of 9.91. The amino acid sequence of the IS*Pso3*-encoded putative Tnp contains the N3 and C1 domains of the invariant DDE motif (Fig. 1) conserved in the IS*4* and IS*5* families (29) with a distance of 49 bp, which is characteristic of the IS*5* family (23). In fact, comparison searches with the ORF1 product in databases revealed homology with Tnps encoded by ISs classified as members of a distinct subgroup of the IS*5* group of the IS*5* family (http://www-is.biotoul.fr/is.html). The highest levels of similarity were observed with Tnps encoded by IS*Ssp126* of *Sphingomonas* sp. strain LB126 (51% identity and 60% similarity) (40), IS*1169* residing in plasmid pIP421 of *Bacteroides fragilis* BF-F238 (approximately 43% identity and 52% similarity) (35), and IS*1168* present in pIP417 of *Bacteroides vulgatus* BV17 (approximately 43% identity and 52% similarity) (17). IS*Pso3* is flanked by identical 11-bp terminal IRs, which exhibit similarity to the IRs of the ISs mentioned above (Fig. 2C). Transposition of this IS resulted in generation of 4-bp DRs (Table 2), a size typical of other elements of the IS*5* group (23).

Occurrence of IS*Pso1*, IS*Pso2*, and IS*Pso3* in different strains of *Paracoccus* spp. Hybridization analysis was performed to study the distribution of the ISs studied in different strains of *Paracoccus* spp. To do this, ISs, PCR performed with cartridge-specific primers (3) were probed with total DNAs of paracocci (listed in Table 1) digested with *Eco*RI and *Pst*I. These restriction enzymes did not cut the ISs analyzed, and therefore the number of hybridized DNA fragments was thought to be equivalent to the minimum number of copies of a given element within the genome.

We detected two copies of IS*Pso1* in parental strain DSM 11592 (Fig. 3A, lane 1) and a single copy of homologous sequences in all of the *P. pantotrophus* strains tested (DSM 65, LMD 82.5, DSM 11072, DSM 11073) (Fig. 3A, lanes 8 to 11). The presence of an IS*Pso1*-like sequence (on restriction fragments that were the same size) in all strains of *P. pantotrophus* strongly suggests that this element was acquired by the common ancestor before branching of these strains. A weak hybridization signal was also observed with a single band of total DNA of *P. versutus* UW1 (Fig. 3A, lane 14), which is phylogenetically closely related to *P. pantotrophus* (1). On the other hand, IS*Pso3* (a member of the IS*5* family) was present only in one strain of *P. pantotrophus* (LMD 82.5) (Fig. 3C, lane 8) in addition to the parental strain (two copies in DSM 11592) (Fig. 3C, lane 1), which suggests that it was recently acquired by a lateral transfer event.

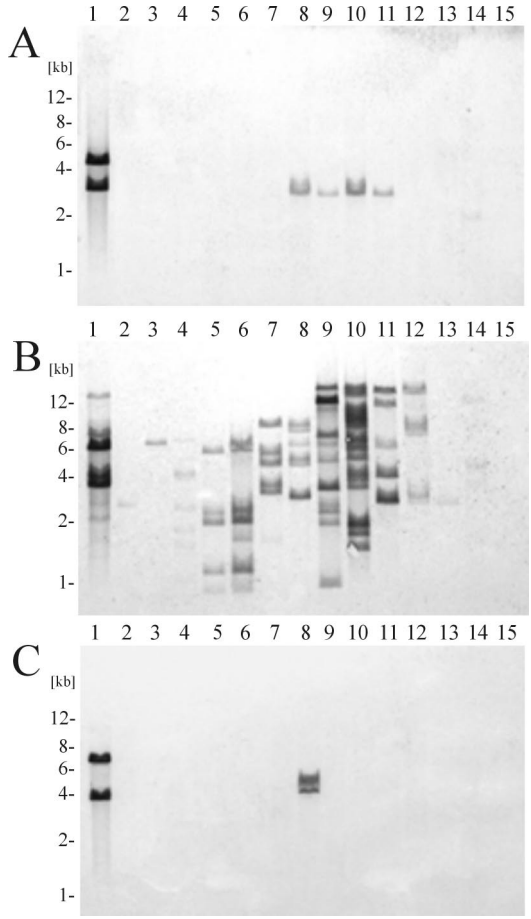

FIG. 3. Analysis of the distribution of IS*Pso1*-like (A), IS*Pso2*-like (B), and IS*Pso3*-like (C) elements by DNA hybridization. The lanes contained *Eco*RI-*Pst*I-digested DNAs of *P. solventivorans* DSM 11592 (lane 1), *P. alkenifer* DSM 11593 (lane 2), *P. aminophilus* JMC 7686 (lane 3), *P. aminovorans* JCM 7685 (lane 4), *P. denitrificans* DSM 413 (lane 5), *P. denitrificans* LMD 22.21 (lane 6), *P. methylutens* DM12 (lane 7), *P. pantotrophus* DSM 65 (lane 8), *P. pantotrophus* LMD 82.5 (lane 9), *P. pantotrophus* DSM 11072 (lane 10), *P. pantotrophus* DSM 11073 (lane 11), *P. alcaliphilus* JCM 7364 (lane 12), *P. thiocyanatus* IAM 12816 (lane 13), *P. versutus* UW1 (lane 14), and pSOV1 of *P. solventivorans* DSM 11592 (lane 15). The positions of the markers are indicated on the left.

Sequences homologous to IS*Pso2* were present in all of the strains of *Paracoccus* spp. tested. The copy number of IS*Pso2* varied from 1 to approximately 14. We observed hybridization with different signal intensities, which reflected the fact that the sequences detected were not identical. As mentioned above, IS*Pso2* and its two closest relatives (IS*Ppa2* and IS*1248*) are highly homologous. These elements do cross-hybridize with each other (3; data not shown), and therefore we were not able to distinguish them by hybridization. In order to study the distribution and diversity of IS*Pso2*-like elements in paracocci,

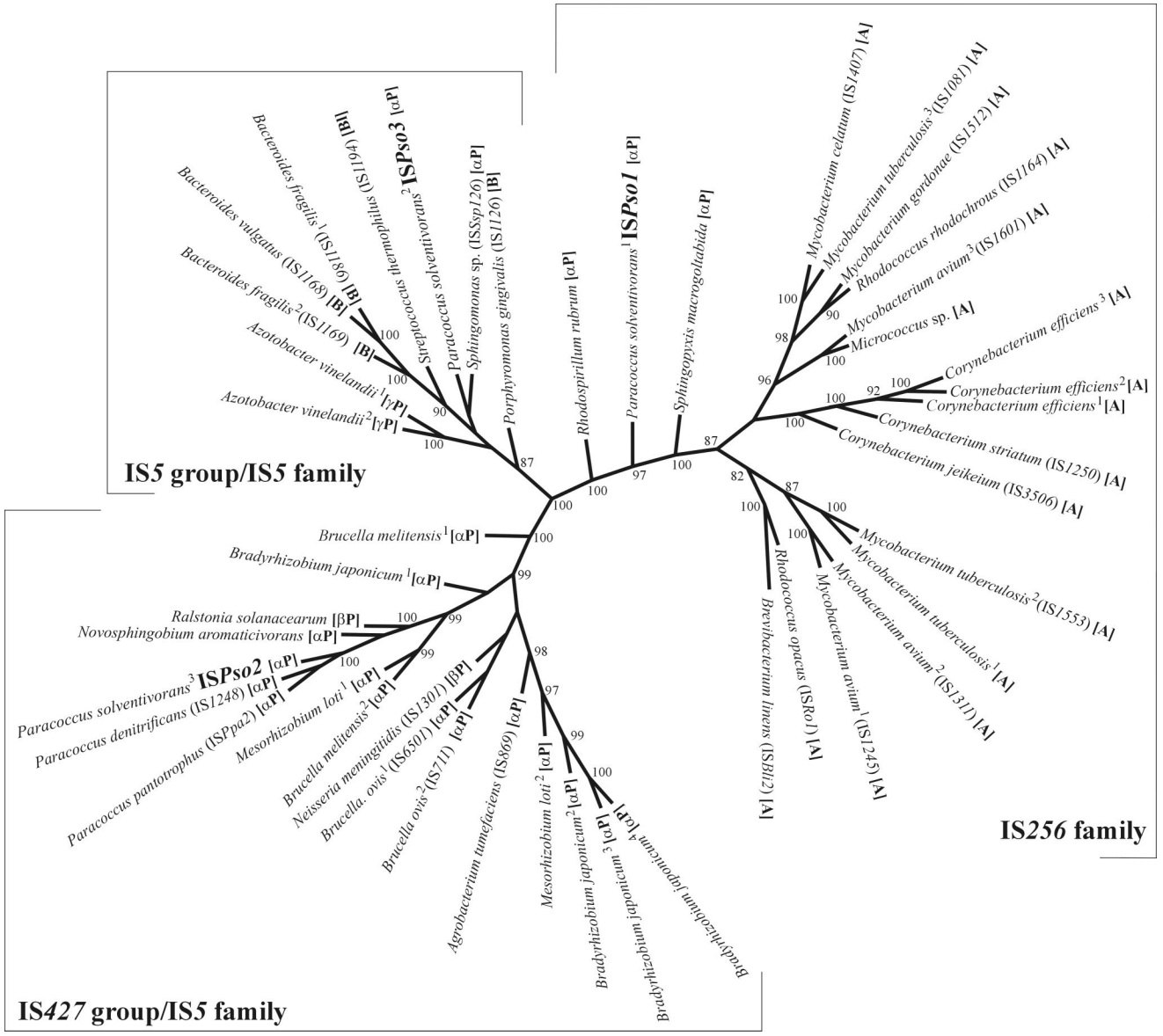

FIG. 4. Unrooted bootstrap tree (1,000 replicates) for putative Tnps encoded by IS*Pso1*, IS*Pso2*, and IS*Pso3* and their closest relatives (identified in available databases), constructed by the parsimony method. The tree was constructed by using SEQBOOT, DNAPARS, CONSENSUS, and DRAWTREE in the software package PHYLIP (14). The numbers at the nodes indicate bootstrap values for the nodes based on 1,000 bootstrap resamplings (values less than 80 are not shown). The taxonomic groups (as described by Garrity and Holt [15]) are indicated in brackets, as follows: A, *Actinobacteria*; B, *Bacteroidetes*; αP, alpha subgroup of the *Proteobacteria*; βP, beta subgroup of the *Proteobacteria*; γP, gamma subgroup of the *Proteobacteria*; Bl, bacilli. The sequences used for this analysis were sequences of *Agrobacterium tumefaciens* (accession no. X53945), *Azotobacter vinelandii* (ZP 00089333 [superscript 1] and ZP 00088382[superscript 2]), *Bacteroides fragilis* (S44507 [superscript 1] and I40184[superscript 2]), *Bacteroides vulgatus* (I40597), *Bradyrhizobium japonicum* (NP 769117 [superscript 1], NP 769117 [superscript 2], NP 771828 [superscript 3], and NP 774822 [superscript 4]), *Brevibacterium linens* (AAF09242), *Brucella melitensis* (NP 540341 [superscript 1] and NP 540319 plus NP 540320 [superscript 2]), *Brucella ovis* (Q08082 [superscript 1] and M94960 [superscript 2]), *Corynebacterium efficiens* (NP 737799 [superscript 1], NP 737110 [superscript 2], and NP 702969 [superscript 3]), *Corynebacterium jeikeium* (NP 848205), *Corynebacterium striatum* (AAG03374), *Mesorhizobium loti* (NP 109452 plus NP 109453 [superscript 1] and NP 108276 [superscript 2]), *Micrococcus* sp. (AAK62483), *Mycobacterium avium* (AAA69904 [superscript 1], CAA11709 [superscript 2], and AAC71696 [superscript 3]), *Mycobacterium celatum* (CAA65977), *Mycobacterium gordonae* (AAB54010), *Mycobacterium tuberculosis* (NP 218157 [superscript 1], NP 338287 [superscript 2], and NP 337078 [superscript 3]), *Neisseria meningitidis* (Z49092), *Novosphingobium aromaticivorans* (ZP 00093225 plus ZP 00093229), *P. denitrificans* (AAC43507 plus AAC43509), *P. pantotrophus* (AAO21198 plus AAO21199), *P. solventivorans* (AAO84921 [superscript 1], AAP76386 [superscript 2], and AAO48787 plus AAO48788 [superscript 3]), *Porphyromonas gingivalis* (CAA10225), *Ralstonia solanacearum* (BAA97979 plus BAA97980), *Rhodococcus opacus* (AAB57888), *Rhodococcus rhodochrous* (BAA11042), *Rhodospirillum rubrum* (ZP 00016443), *Sphingomonas* sp. (CAB87573), *Sphingopyxis macrogoltabida* (BAB07803), and *Streptococcus thermophilus* (AF454495). Some of the Tnps of the IS*427* group are encoded by two overlapping ORFs; therefore, two accession numbers are given above. In these cases the sequences of the transframe fusion Tnps (generated in silico within the putative frameshift motif) were used for the analysis. The designations of the defined ISs are the GenBank and ISDatabase designations.

we designed specific primers for each of the three related elements (the nucleotide sequences of the primers are given in Materials and Methods) and used them (together with total DNAs of the strains analyzed) in a PCR analysis. The results obtained showed that these elements are highly divergent. We did not observe PCR amplification in the majority of the paracoccal species tested (Table 1). We were able to detect IS*Pso2* only in parental strain DSM 11592 and in *P. pantotrophus* LMD 82.5. IS*1248* was detected in all strains of *P. pantotrophus* and *P. denitrificans*, as well as in *P. methylutens* DM12, while IS*Ppa2* was present in all but one strain (LMD 82.5) of *P. pantotrophus* and in *P. methylutens* DM12. The simultaneous presence of IS*Ppa2* and IS*1248* in some strains (Table 1) is not surprising since it was previously shown that IS*Ppa2*-like elements are harbored by related plasmids of two strains of *P. pantotrophus* (DSM 11073 and DSM 65) (3), which suggests the possibility that they were disseminated by lateral transfer. However, the possibility that different copies of the ancestor element evolved divergently in the same host cannot be eliminated.

None of the ISs identified hybridized with the only natural plasmid (2) harbored by *P. solventivorans* DSM 11592, plasmid pSOV1 (Fig. 3, lane 15); therefore, all of them reside in the chromosome of this strain.

Phylogenetic analysis. Comparisons of the Tnps encoded by IS*Pso1*, IS*Pso2*, and IS*Pso3* in databases allowed identification of their closest homologues. These sequences were used for construction of a phylogenetic tree, which showed the evolutionary relationships (Fig. 4) and allowed estimation of possible directions of lateral transfer of transposable elements between various bacterial hosts. As expected, the phylogenetic analysis separated the Tnps into three subgroups (comprising sequences with similarity to IS*256* products and products of two groups belonging to the IS*5* family), which were well supported by bootstrap values (Fig. 4).

As Fig. 4 shows, the Tnp encoded by IS*Pso2* is closely related to a number of Tnps produced by bacteria belonging to the *Proteobacteria*, the majority of which (all but two) are classified (like paracocci) in the alpha subgroup of the *Proteobacteria*. This suggests that a unique transfer event might have occurred in a putative progenitor of bacteria belonging to this class. The widespread distribution of these elements in paracocci (as shown by hybridization analysis [Fig. 3B]) seems to support this hypothesis. The presence within the paracoccal cluster of a closely related Tnp of *Ralstonia solanacearum* (a member of the beta subgroup of the *Proteobacteria*) (Fig. 4) suggests, however, that both common ancestry and lateral transfer are part of the evolutionary history of these ISs.

Interestingly, Tnps of the IS*Pso1* type were found exclusively in bacteria belonging to two phylogenetically unrelated taxa, the alpha subgroup of the *Proteobacteria* and the *Actinobacteria*. The IS*Pso1* sequence has a high G+C content (Table 1), which is typical of both groups of bacteria. The Tnps of members of the alpha subgroup of the *Proteobacteria* (*P. solventivorans*, *Rhodospirillum rubrum*, and *Sphingopyxix macrogoltabida*, which are classified in different orders) do not form a separate cluster, which might suggest that they were acquired by different transfer events.

The IS*Pso3*-like Tnps, located on the same branch of the phylogenetic tree (Fig. 4), are produced by members of different groups of bacteria (including the gram-negative alpha and gamma subgroups of the *Proteobacteria* and *Bacteroidetes*, as well as gram-positive bacilli), indicating their broad host range and frequent transfer between various bacterial genera.

The ISs described in this paper are the first transposable elements identified in *P. solventivorans*. Some of these elements might be useful for strain or species identification. These elements are members of two different families, the IS*256* family (IS*Pso1*) and the IS*5* family (IS*Pso2* and IS*Pso3*). Several elements classified in the IS*5* family (which is relatively heterogeneous and comprises several groups) were also identified in *P. pantotrophus* (3), which suggests that ISs belonging to this family predominate in paracocci.

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REFERENCES

1. **Baj, J.** 2000. Taxonomy of the genus *Paracoccus.* Acta Microbiol. Pol. **49:** 185–200.
2. **Baj, J., E. Piechucka, D. Bartosik, and M. Wlodarczyk.** 2000. Plasmid occurrence and diversity in the genus *Paracoccus.* Acta Microbiol. Pol. **49:**265–270.
3. **Bartosik, D., M. Sochacka, and J. Baj.** 2003. Identification and characterization of transposable elements of *Paracoccus pantotrophus.* J. Bacteriol. **185:**3753–3763.
4. **Beijerinck, M., and D. C. J. Minkman.** 1910. Bildung und Verbrauch von Stickoxydul durch Bakterien. Zentralbl. Bakteriol. Parasitenkd. Infektionsk. **25:**30–63.
5. **Berry, A., D. Janssens, M. Humbelin, J. P. Jore, B. Hoste, I. Cleenwerck, M. Vancanneyt, W. Bretzel, A. F. Mayer, R. Lopez-Ulibarri, B. Shanmugam, J. Swings, and L. Pasamontes.** 2003. *Paracoccus zeaxanthinifaciens* sp. nov., a zeaxanthin-producing bacterium. Int. J. Syst. Evol. Microbiol. **53:**231–238.
6. **Birnboim, H. C., and J. Doly.** 1979. A rapid alkaline extraction procedure for screening recombinant plasmid DNA. Nucleic Acids Res. **7:**1513–1519.
7. **Chandler, M., and J. Mahillon.** 2002. Insertion sequences revised, p. 305–366. *In* N. L. Craig, R. Craigie, M. Gellert, and A. M. Lambowitz (ed.), Mobile DNA II. American Society for Microbiology, Washington, D.C.
8. **Daneshvar, M. I., D. G. Hollis, R. S. Weyant, A. G. Steigerwalt, A. M. Whitney, M. P. Douglas, J. P. Macgregor, J. G. Jordan, L. W. Mayer, S. M. Rassouli, W. Barchet, C. Munro, L. Shuttleworth, and K. Bernard.** 2003. *Paracoccus yeeii* sp. nov. (formerly CDC group EO-2), a novel bacterial species associated with human infection. J. Clin. Microbiol. **41:**1289–1294.
9. **Devereux, J., P. Haeberli, and O. Smithies.** 1984. A comprehensive set of sequence analysis programs for the VAX. Nucleic Acids Res. **11:**387–395.
10. **Ditta, G., S. Stanfield, D. Corbin, and D. R. Helinski.** 1980. Broad host-range DNA cloning system for gram-negative bacteria: construction of a gene bank of *Rhizobium meliloti.* Proc. Natl. Acad. Sci. USA **77:**7347–7351.
11. **Doronina, N. V., Y. A. Trotsenko, B. B. Kuznetzov, and T. P. Tourova.** 2002. Emended description of *Paracoccus kondratievae.* Int. J. Syst. Evol. Microbiol. **52:**679–682.
12. **Doronina, N. V., Y. A. Trotsenko, V. I. Krausowa, and N. E. Suzina.** 1998. *Paracoccus methylutens* sp. nov.—a new aerobic facultatively methylotrophic bacterium utilizing dichloromethane. Syst. Appl. Microbiol. **21:**230–236.
13. **Escoubas, J. M., M. F. Prere, O. Fayet, I. Salvignol, D. Galas, D. Zerbib, and M. Chandler.** 1991. Translational control of transposition activity of the bacterial insertion sequence IS*1*. EMBO J. **10:**705–712.
14. **Felsenstein, J.** 1989. PHYLIP, phylogenetic inference package (version 3.2). Cladistics **5:**164–166.
15. **Garrity, G. M., and J. G. Holt.** 2001. The road map to the manual, p. 119–166. *In* G. M. Garrity (ed.), Bergey's manual of systematic bacteriology, vol. 1. Springer-Verlag, New York, N.Y.
16. **Haas, M., and B. Rak.** 2002. *Escherichia coli* insertion sequence IS*150*: transposition via circular and linear intermediates. J. Bacteriol. **184:**5833–5841.
17. **Haggoud, A., G. Reysset, H. Azeddoug, and M. Sebald.** 1994. Nucleotide sequence analysis of two 5-nitroimidazole resistance determinants from *Bacteroides* strains and of a new insertion sequence upstream of the two genes. Antimicrob. Agents Chemother. **38:**1047–1051.
18. **Jordan, S. L., I. R. McDonald, A. J. Kraczkiewicz-Dowjat, D. P. Kelly, F. A. Rainey, J. C. Murrell, and A. P. Wood.** 1997. Autotrophic growth on carbon disulfide is a property of novel strains of *Paracoccus denitrificans.* Arch. Microbiol. **168:**225–236.
19. **Katayama, Y., A. Hirashi, and H. Kuraishi.** 1995. *Paracoccus thiocyanatus*

sp. nov., a new species of thiocyanate-utilizing facultative chemolithotroph, and transfer of *Thiobacillus versutus* to the genus *Paracoccus* as *Paracoccus versutus* comb. nov. with emendation of the genus. Microbiology **141:**1469–1477.

20. **Kelly, D. P., F. A. Rainey, and A. P. Wood.** December 2000, posting date. The genus *Paracoccus*. *In* M. Dworkin, S. Falkow, E. Rosenberg, K. H. Schleifer, and E. Stackebrandt (ed.), The prokaryotes, 3rd ed. [Online.] Springer Verlag, New York, N.Y. http://link.springer-ny.com/link/service/books/10125/.
21. **Kushner, S. R.** 1978. An improved method for transformation of *E. coli* with ColE1-derived plasmids, p. 17–23. *In* H. B. Boyer and S. Nicosia (ed.), Genetic engineering. Elsevier/North-Holland, Amsterdam, The Netherlands.
22. **Lipski, A., K. Reichert, B. Reuter, C. Spröer, and K. Altendorf.** 1998. Identification of bacterial isolates from biofilters as *Paracoccus alkenifer* sp. nov. and *Paracoccus solventivorans* with emended description of *Paracoccus solventivorans.* Int. J. Syst. Bacteriol. **48:**529–536.
23. **Mahillon, J., and M. Chandler.** 1998. Insertion sequences. Microbiol. Mol. Biol. Rev. **62:**725–774.
24. **Merlin, C., J. Mahillon, J. Nesvera, and A. Toussaint.** 2000. Gene recruiters and transporters: the modular structure of bacterial mobile elements, p. 363–409. *In* C. M. Thomas (ed.), Horizontal gene pool. Harwood Academic Publishers, Amsterdam, The Netherlands.
25. **Neef, A., A. Zaglauer, H. Meier, R. Amann, H. Lemmer, and K. H. Schleifer.** 1996. Population analysis in a denitrifying sand filter: conventional and in situ identification of *Paracoccus* spp. in methanol-fed biofilms. Appl. Environ. Microbiol. **62:**4329–4339.
26. **Ohara, M., Y. Katayama, M. Tsuzaki, S. Nakamoto, and H. Kuraishi.** 1990. *Paracoccus kocurii* sp. nov., a tetramethylammonium-assimilating bacterium. Int. J. Syst. Bacteriol. **40:**292–296.
27. **Pukall, R., M. Laroche, R. M. Kroppenstedt, P. Schumann, E. Stackebrandt, and R. Ulber.** 2003. *Paracoccus seriniphilus* sp. nov., an L-serine-dehydratase-producing coccus isolated from the marine bryozoan *Bugula plumosa.* Int. J. Syst. Evol. Microbiol. **53:**443–447.
28. **Rainey, F. A., D. P. Kelly, E. Stackebrandt, J. Burghardt, A. Hiraishi, Y. Katayama, and A. P. Wood.** 1999. A re-evaluation of the taxonomy of *Paracoccus denitrificans* and a proposal for the combination *Paracoccus pantotrophus* comb. nov. Int. J. Syst. Bacteriol. **49:**645–651.
29. **Rezsohazy, R., B. Hallet, J. Delcour, and J. Mahillon.** 1993. The IS*4* family of insertion sequences: evidence for a conserved transposase motif. Mol. Microbiol. **9:**1283–1295.
30. **Roberston, L. A., and J. G. Kuenen.** 1983. *Thiosphaera pantotropha* gen. nov. sp. nov., a facultative anaerobic, facultatively autotrophic sulphur bacterium. J. Gen. Microbiol. **129:**2847–2855.
31. **Sambrook, J., and D. W. Russel.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
32. **Schneider, D., D. Faure, M. Noirclerc-Savoye, A. C. Barrière, E. Coursange, and M. Blot.** 2000. A broad-host-range plasmid for isolating mobile genetic elements in gram-negative bacteria. Plasmid **44:**201–207.
33. **Siller, H., F. A. Rainey, E. Stackebrandt, and J. Winter.** 1996. Isolation and characterization of a new gram-negative, acetone-degrading, nitrate-reducing bacterium from soil, *Paracoccus solventivorans* sp. nov. Int. J. Syst. Bacteriol. **46:**1125–1130.
34. **Taylor, B., and D. S. Hoare.** 1969. New facultative *Thiobacillus* and a reevaluation of the heterotrophic potential of *Thiobacillus novellus.* J. Bacteriol. **100:**487–497.
35. **Trinh, S., A. Haggoud, G. Reysset, and M. Sebald.** 1995. Plasmids pIP419 and pIP421 from *Bacteroides*: 5-nitroimidazole resistance genes and their upstream insertion sequence elements. Microbiology **141:**927–935.
36. **Tsubokura, A., H. Yoneda, and H. Mizuta.** 1999. *Paracoccus carotinifaciens* sp. nov., a new aerobic gram-negative astaxanthin-producing bacterium. Int. J. Syst. Bacteriol. **49:**277–282.
37. **Urakami, T., H. Araki, H. Oyanagi, K. Suzuki, and K. Komagata.** 1990. *Paracoccus aminophilus* sp. nov. and *Paracoccus aminovorans* sp. nov., which utilize *N*,*N*-dimethylformamide. Int. J. Syst. Bacteriol. **40:**287–291.
38. **Urakami, T., J. Tamaoka, K. Suzuki, and K. Komagata.** 1989. *Paracoccus alcaliphilus* sp. nov., an alkaliphilic and facultatively methylotrophic bacterium. Int. J. Syst. Bacteriol. **39:**116–121.
39. **Van Spanning, R. J. M., A. P. De Boer, D. J. Slotboom, W. N. Reijnders, and A. H. Stouthamer.** 1995. Isolation and characterization of a novel insertion sequence element, IS*1248*, in *Paracoccus denitrificans.* Plasmid **34:**11–21.
40. **Wattiau, P., L. Bastiaens, R. van Herwijnen, L. Daal, J. R. Parsons, M. E. Renard, D. Springael, and G. R. Cornelis.** 2001. Fluorene degradation by *Sphingomonas* sp. LB126 proceeds through protocatechuic acid: a genetic analysis. Res. Microbiol. **152:**861–872.
41. **Zheng, J., and M. A. McIntosh.** 1995. Characterization of IS*1221* from *Mycoplasma hyorhinis*: expression of its putative transposase in *Escherichia coli* incorporates a ribosomal frameshift mechanism. Mol. Microbiol. **16:**669–685.